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Collaborative study to assess the suitability of the candidate WHO International Reference Reagent for MSC identity (for flow cytometry) for advanced therapies.

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** See Appendix*

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **27 September 2019** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

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Summary

We report the results of a collaborative study that was conducted with the aim to asses a reference reagent for mesenchymal stem cell identity for flow cytometry. Currently, there are no standards available for mesenchymal stem cells. 75 vials were produced. The vials are stable for 6 months at -20 degrees. 15 participants from 9 different laboratories took part. The collaborators were asked to run the reagent in their inhouse flow cytometry set-up as usual, and report back as if it was one of their own samples. No restrictions or recommendations were given.

The collaborative study showed that the reference reagent performed extremely well under all the different conditions. The mean values from this study fall very close to the ranges for % expression for each of the markers in the ISCT recommendations for MSC identity, and it was possible to generate a range for each marker that was +/- 2SD. The reagent is not a replacement for the ISCT values but a tool to help researchers to validate their equipment and results. In addition, this pilot study allowed us to identify issues around the development and establishment of novel reference reagents for advanced therapies.

Introduction

MSCs (alternatively called mesenchymal stem cells, multipotent stromal cells or mesenchymal stromal cells) are a type of multipotent adult stem-like cell possessing unique regenerative and immunomodulatory abilities that have propelled them into the cellular therapy spotlight. Currently, there are over a hundred clinical trials (clinical trials.gov) involving either MSCs or MSC derived products recruiting patients.

In 2006, the International Society of Cellular Therapies (ISCT) issued a series of minimal criteria to define MSCs (Dominici, 2006). These included that cells must express high levels (≥95%) of CD105, CD73 and CD90 whilst lacking expression (≤2%) of CD45, CD34, CD14, CD11b, CD19 and HLA-DR surface proteins, confirmation of which is achieved through flow cytometry. However, a survey by Trento (Trento, 2016) highlighted the disarray within the MSC field when it comes to characterisation and testing to define MSC populations. This is further compounded by the inherent variation seen in flow cytometry due to biological variability of cells, limited stability of samples and different requirements for cytometer setup and data interpretation. Together, these factors have made it difficult to compare different MSC based products both within (batch to batch) and across laboratories.

In 2018, the WHO expert committee on Biological Standardization (ECBS) endorsed from the National Institute for Biological Standards and Control (NIBSC) the proposal to develop a WHO Reference Reagent to serve as a reference reagent for flow cytometry identity of MSC populations.

The proposed reference reagent can be used as a reference material for flow cytometry identity assessment of MSC products and will facilitate the validation and verification of the identity of MSC populations used in clinical applications. The reagent can be used to identify technical issues, to perform batch to batch analysis and can add confidence to data.

Bulk materials, processing and characterization

Material (15/270) was prepared from the human iPSC line NIBSC_i008 (N8). Briefly, lung fibroblast cells were reprogrammed using mRNA to create the stable N8 cell line. Subsequent differentiation into MSCs was achieved by culturing N8 cells in DMEM supplemented with 10% FCS and 10µM SB431542 for 10 days (Chen, 2012). Serial passaging into non-coated tissue culture flasks was then performed and fully differentiated MSCs were observed at passage 4. At this point cell samples were taken for quality control (QC) analysis via flow cytometry to assess expression of MSC specific markers outlined by the ISCT guidelines. Two additional markers, CD45 and CD29 were included because they are often characterized on MSC preparations (reference). Since this material is intended for use in flow cytometry studies, no additional characterization of this MSC line is reported.

Following confirmation of the correct marker profile, N8-MSC was sub cultured and expanded in MesenCult™ media, a standardised, serum containing medium for MSC growth. When enough cell numbers had been reached, cells were collected, and a sample taken for repeat flow cytometry to confirm retainment of surface marker expression. Cells were then resuspended in MesenCult™ containing fixative. Fixed cells were stored at 4°C overnight. Finally, cells were washed and re suspended in freeze-drying formulation and distributed into ampoules (approximately $1x10^6$ cells per ampoule). The ampoule contents were freeze dried and sealed under nitrogen. The finished product characteristics are shown in table 1. 75 vials were generated.

Table 1: Characteristics of freeze-dried reference reagent.

Code Number	18/212-001
Presentation-	Sealed, 5ml glass ampoules
Number of ampoules produced	75
Date Filled	January 2019
Mean fill mass (g)	1.142
CV of fill mass (%)	0.2
Residual Moisture	N/A
Mean Dry weight	N/A
Mean Oxygen head space	N/A
Microbiological results	N/A
Storage Conditions	-20 degrees
Address of processing facility	NIBSC, Potters Bar, EN6 3QG, UK
Address of Custodian	NIBSC, Potters Bar, EN6 3QG, UK

Quality Control (QC) of freeze dried MSC reference reagent

Five ampoules were randomly selected and tested at NIBSC for consistency and compared to a commercial MSC line isolated from bone marrow (BM) and a line from human umbilical cord (UC). Human neural stem cells were run as a negative control. Vials were reconstituted in 1ml of PBS and tested for MSC markers CD90, CD44, CD105 and CD73 (BD Bioscience Kit Cat: 562245) and negative markers CD34, CD11b, CD19, CD45 and HLA-DR (negative cocktail from BD Bioscience Kit Cat: 562245) Flow cytometry was performed using the BD Accuri according to manufactures instructions. The results can be seen in Figure 1.

The reference reagent showed extremely strong repeatability in expression of markers between the vials.

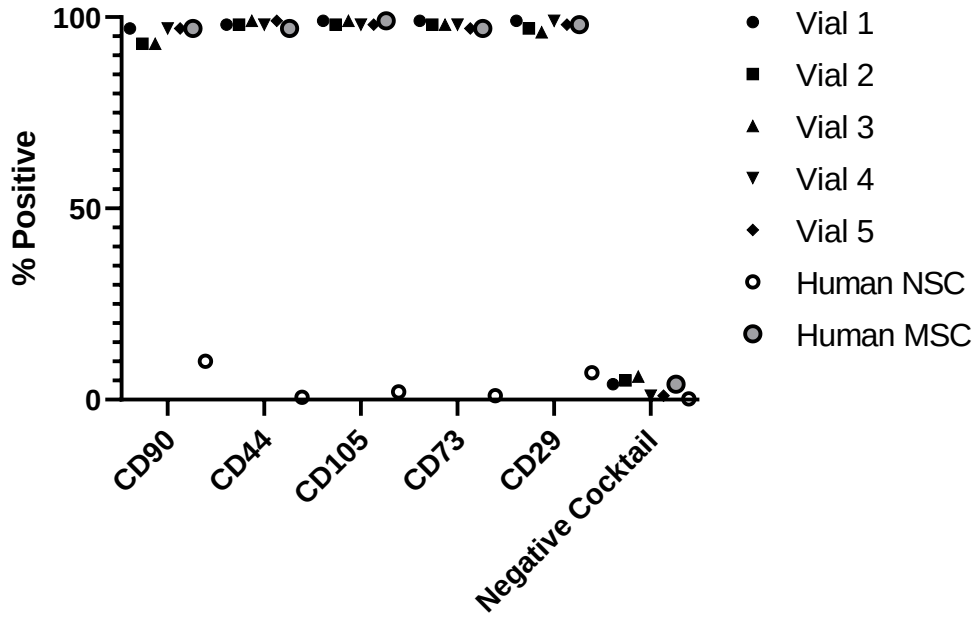


Figure 1: The percentage of positive cells for each sample for an antibody specific marker for the reference reagent, as measured on site in NIBSC. Human MSCs (Bone Marrow) and human Neural Stem Cells (NSCs) were run as positive and negative controls respectively. The average and SD values in the table are based on the MSC standard vials only.

Collaborative study

The collaborative study was organised by NIBSC. 15 participants from 9 different countries kindly agreed to take part. The aim of the study was to see who the vials behaved under untested conditions in different labs. Three ampoules were provided to each participant, and each laboratory was asked to perform their in-house method for flow cytometry, using their preferred antibodies and protocols for MSC analysis. The labs were purposely not given any

protocols or standard operating procedures (SOPs) as this study was to determine the suitably of the reference reagent under any local conditions (e.g. staining procedures, fluorochromes/antibodies, gating and analysis, flow cytometer). The only instructions given were on how to reconstitute the MSC vials.

The participants were asked to run the standard as per normal procedures, i.e. as if they were running an MSC sample themselves. This included free choice of antibody, flow cytometer and data analysis software.

Participants

The participants are listed in Appendix 1, alphabetically by country. The participants include a mixture of academic and industry research groups with extensive experience in MSC biology and characterisation. Each participating laboratory is referred to in the study by a code number. The code numbers were randomly assigned and do not reflect the order of listing.

Results and data analysis

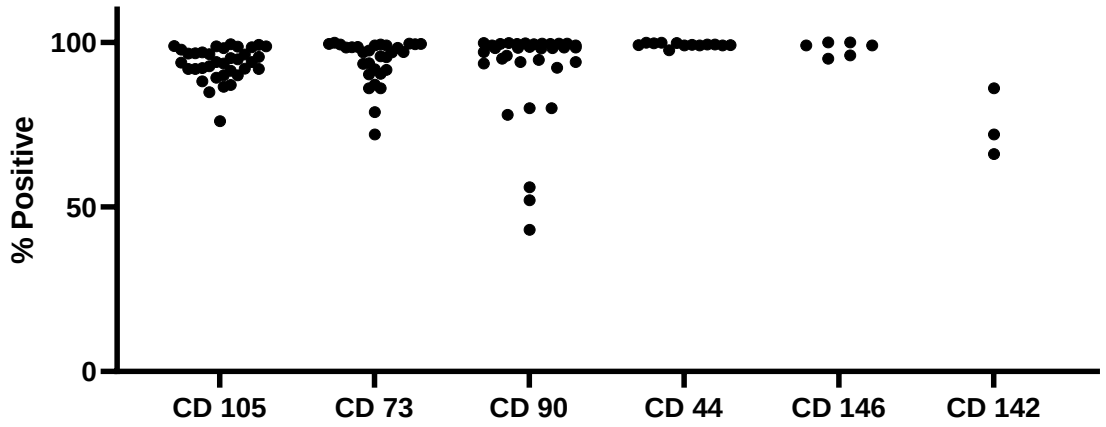
Data returned for analysis
Of the 15 laboratories sent material, data was returned by 13 laboratories who performed their routine MSC characterisation on the standard. Laboratory 5 and 14 could not return the data back in the agreed time-frame. Data from laboratory 12 was unusable due to low cell number analysed and was not included. Laboratory 4 received one empty vial and was excluded.

General suitability of the reference material
Each laboratory reported back the percent of positive cells for each marker they ran, for both positive and negative markers for MSCs. Each laboratory ran their vials as three separate samples. The list of antibodies, flow cytometers and analysis software can be found in Appendix 2.

Positive Markers

Each of the 11 laboratories tested the recommended ISCT positive markers (CD105, CD73 and CD90). 5 laboratories also used CD44, and two laboratories tested CD146 and one tested at CD142. The mean percent positive cells and standard deviation can be seen in the table in Figure 2.

One laboratory tested CD271 which is bone marrow specific (Álvarez-Viejo, 2015). The standard was negative for the marker as it is not from bone marrow

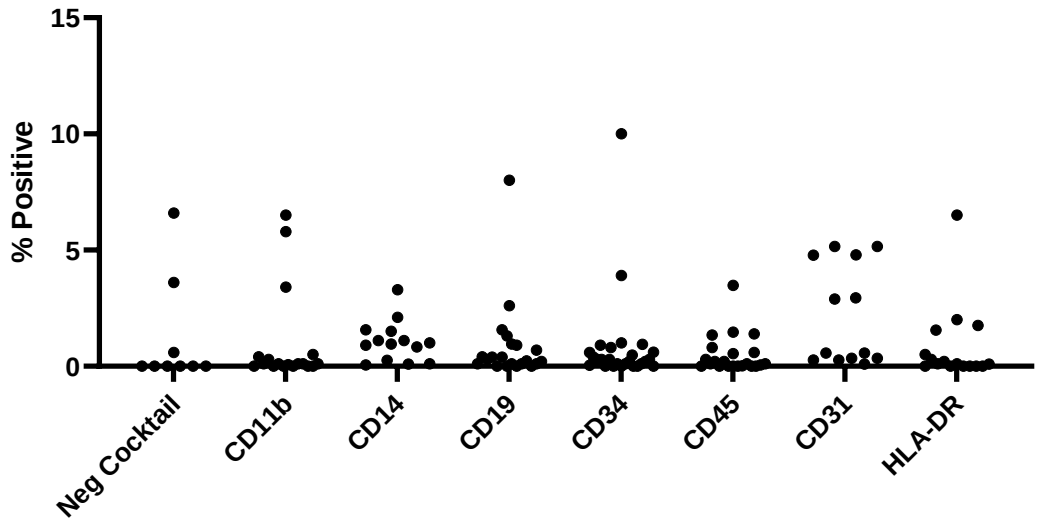


	CD105	CD73	CD90	CD44	CD146	CD142
Mean	93.4	94.3	94.5	99	98	74
SD	4.7	5.8	6.4	0.4	2.1	
N (labs) =	11	11	10*	5	2	1

Figure 2: The percentage of positive cells for each marker from participants for antibody specific markers expected to be positive for the reference reagent. *One lab was excluded from analysis as the data was over half the values of the average.

Negative Markers

Not all laboratories tested all the recommended ISCT markers. One laboratory had a highly positive result for CD45, and this was excluded. The reference reagent was strongly negative for all frequently used negative MSC markers that were tested by the labs. Not shown in the graph, one lab also tested the reference reagent for CD80.



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	Neg Cocktail	**CD11b**	**CD14**	**CD19**	**CD34**	**CD45**	**CD31**	**HLA-DR**
Mean	1.2	0.9	1	0.9	0.7	0.3	1.8	0.8
SD	2.3	2	0.8	2	1.9	0.7	2.1	1.6
N(labs) =	3	9	6	7	9	*10	3	6

Figure 3: The % positive for each marker from participants for an antibody specific marker for the reference reagent. *One lab removed for positive result.

Range of values

To calculate the range of expected values for each marker (based on the results obtained in this study), the highest and lowest scoring labs were removed (when n>10), and the range was calculated as +/- 2SD**.**

Positive Markers

	CD105	CD73	CD90	CD44	CD142	CD146
Mean	93.32	94.15	95.79	99.22	74.67	98.30
SD	2.72	3.17	4.27	0.41		
N(labs)	9	9	8	5	1	2
Mean-2SD	87.87	87.82	87.25	98.40		
Mean+2SD	98.77	100	100	100		

Negative Markers

	Neg Cocktail	CD11b	CD14	CD19	CD34	CD45	CD31	CD80	HLA-DR
Mean	1.2	0.9	1	0.9	0.7	0.3	1.8	14	0.8
SD	2.3	2	0.8	2	1.9	0.7	2.1	4	1.6
N(labs) =	3	9	6	7	9	10	3	1	6
Mean-2SD	0	0.4	0	0.5	0	0	0		0
Mean+2SD	5.8	4.9	2.6	4.9	4.3	1.7	6		4

Figure 4: Data table with range +/-2SD of the mean for positive and negative MSC markers

Stability study

Because this was a pilot study, the batch size produced for this standard restricts the extent to which stability can be assessed. This batch has been tested after 6 months at -20°C and the reagent is stable (Figure 5). After reconstitution in PBS, the reagent is stable at 4°C for 24 hours (Figure 6).

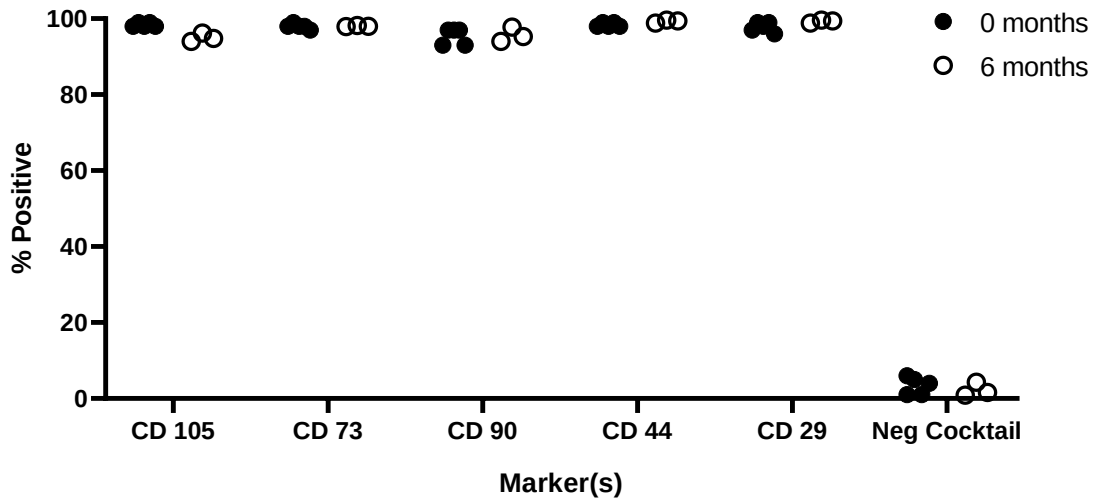

Figure 5: Stability of reference reagent after 6 months at -20°C.

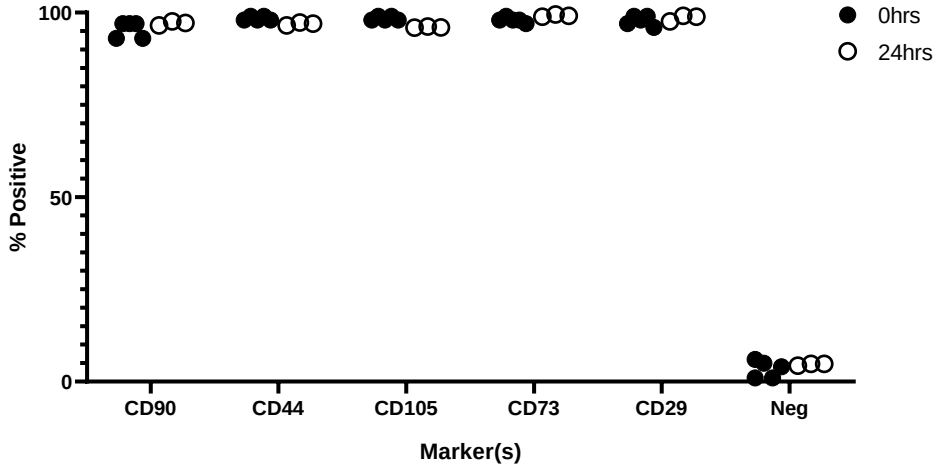

Figure 6: Stability of reference reagent after reconstitution in PBS and left at 4°C for 24 hours.

Comments from Participants

A draft report was circulated to all participating laboratories of the study for comments. There were no objections. All minor comments can be found in appendix 3.

Discussion

A reference reagent is only useful to labs when it works within their in-house system. Furthermore, the nature of flow cytometry makes it a difficult technique to standardize as it relies on self-instrument settings and data analysis, increasing the likelihood of discrepancies amongst different groups. This is where the reference reagent can help. This study was purposefully designed to test the reference reagent under any potential mixture of antibody, machinery, analysis and protocols.

In this study no two labs ran the same antibodies, flow cytometer machine or analysis software. All labs were given free choice of gating strategies also. Despite this, the reference reagent performed extremely well across all the conditions and there was generally good agreement between laboratories with low SD for both the positive and negative markers. This study has highlighted the flexibility and robustness of the reference reagent. There was no combination where it did not perform as expected. There were some outliers, and these are currently being investigated with the relevant labs.

The mean values from this study fall very close to the ranges for % expression for each of the markers in the ISCT recommendations for MSCs *(≥95% of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. Additionally, these cells must lack expression (≤ 2% positive) of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II).* The reagent is not a replacement for the ISCT values but a tool to help researchers to validate their results.

It is important to emphasize that the proposed reference material is not intended to be a gold standard, which are defined by a stringent set of rules such as those outlined by the ISCT.

Instead, we propose that this reference reagent as a material to be used as a mechanism to validate set up and results. This is particularly critical in a technique such as flow cytometry which relies on self-user instrument set up and data analysis. This along with the vast range of instruments, software and antibodies/fluorochromes inevitably lead to data variation. The existence of such a reference material would provide users with a quality control check, assuring that their assay is working and thus the data obtained for their unknown samples can be trusted.

There was some considerable lab to lab variation in marker score, for example, labs 1, 7 and 12 scored almost identical to the standard when compared to the vials tested as NIBSC, despite different antibodies, cytometers, and analysis. Whereas labs 8 and 9, although scored high, were over 10% out in some values for some markers. This is the main factor for the variation in SD range.

One lab reported difficulty with when opening the glass vials and reconstituting the sample. A solution to this is we could change the glass vials to wide neck plastic ones, but we would need to check this didn't affect the performance of the standard. Two labs noted problems with low cell numbers when it came to running samples. A solution to this is moving forward, would be to provide a more detailed protocol for reconstituting the cells. One lab reported that the standard helped to identify an internal technical issue, emphasizing the value of this reference reagent as a tool to help labs be confident that their flow cytometry system is working.

Overall, our results indicate that this standard is fit for purpose, the expression of MSC associated markers are independent of the flow cytometer system used as evidenced by the results of this report, furthermore technical replicates within each individual lab showed high consistency. Thus, for this reference reagent we propose that positive markers for CD105, CD73, CD90 and CD44 should ideally be close to the mean and not outside the 2SD range for each individual value. Within this range, the reagent can give confidence that your assay is working. If these limits are breached it would indicate that the assay may be out of specification and further investigation would be needed.

As the negative cocktail contains several antibodies, and compensation is often required, the value was slightly higher (1%) than for individual markers.

This is especially useful for the analysis of MSCs whereby the presence/absence of the ISCT markers are sensitive to tissue source, culture conditions, passage number etc. The reference reagent would provide users with the confidence that the marker expression they see in their MSC samples are true biological results and have not been influenced during the assay set up process.

<u>Points to consider</u>

1. This collaborative study was a proof of principal trial, as we were unsure of how the reagent would perform under any mixture of conditions (choice of antibody, cytometer, gating etc). Therefore, a small pilot batch was produced and all vials have been used for internal studies or within the collaborative study.

2. It should be noted that due to the biological nature of such a material, its shelf-life will be much shorter compared to regular WHO standards; therefore, production of bigger batches might be ineffective.
3. Due to current manufacturing facilities and staff constrains, the current batch size that can be produced is limited to approximately 100 vials. If increased numbers are required, new manufacturing capabilities will need to be evaluated.
4. Alternatively, consideration could be given to performing batch-to-batch comparisons, with smaller collaborative groups (3-5) for subsequent batches.

Overall, we consider this proof of principal trial to have been very successful and crucial to highlight the benefits and manufacturing issues of such novel reference materials. Particularly, in order to proceed with the establishment of reference reagent for distribution, a new batch would need to be generated for distribution and compared to this one, with 2 alternative approaches (Fig. 7). One option would be to evaluate scale-up mechanisms to produce a bigger batch and repeat the collaborative study (Fig. 7A). This approach would allow production of a reference material within the current accepted parameters; however, it might not be efficient both in what regards to the biological nature of the product as well as the time that its implementation would take. Another option would be to perform batch-to-batch comparisons and a smaller collaborative study (3-5 groups) (Fig. 7B). Since all batches would be produced from the same master bank, one could assume that the batches behave very similarly if they have the same identity profile. This option would allow continuing using the current manufacturing process; however, it should be noted that this may or may not affect the range value proposed here.

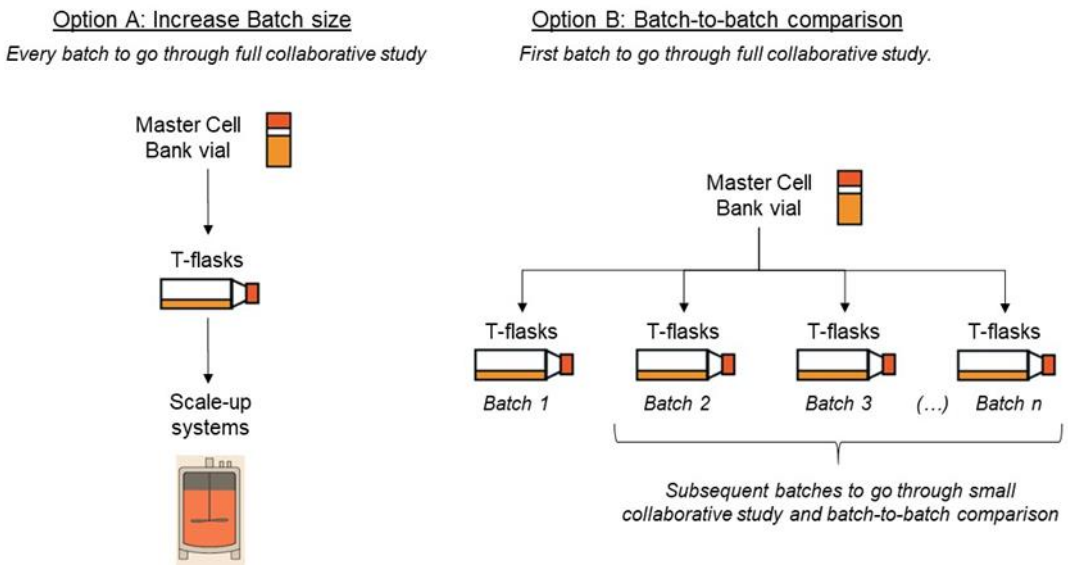

Figure 7. Proposed options for addressing manufacturing constrains and biological nature of novel reference reagents. A) Production of bigger batch sizes would require evaluation of manufacturing systems that allow for scale-up. B) Initial batch produced from master cell bank to be evaluated with full collaborative study, while subsequent batches evaluation would rely on smaller collaborative studies and comparability studies.

Proposal for WHO

For this reference reagent batch, it is proposed that positive markers CD105, CD73, CD90 and CD44 should ideally be close to the mean and not outside the 2SD range for each individual value. For negative markers it is proposed that individual the standards should not score >5%, and with a neg cocktail >6%. Any value higher than this may indicate the assay may be out of specification. Within this range, the reagent can give confidence that your assay is working.

Acknowledgements

We gratefully acknowledge the important contributions of all the participants in the collaborative study, and the Centre for Biological Reference Materials, NIBSC for preparation and dispatch of the ampoule materials.

References

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Chen et al, *Stem Cells Translational medicine*, 2012;1:83–95

Dominici et al, *Cytotherapy*. 2013 Jan;15(1):2-8. doi: 10.1016/j.jcyt.2012.10.002.

Trento et al, *Blood, Volume 128(22):3374-3374, December 2, 2016*

Appendix 1 List of participants alphabetically by order of country

Canada	Krembil Research Institute Professor Sowmya Viswanathan/ Dr. Shrinidh Joshi
Germany	Charité Universitätsmedizin Berlin/BCRT Dr Guido Moll/ Dr. Mathias Streitz/ Dr. Stephan Schlickeiser
Ireland	Regenerative Medicine Institute, National University of Ireland Professor Frank Barry/ Dr. Nahidul Islam
Portugal	Stem Cell Engineering Research Group, iBB-Institute for Bioengineering and Biosciences Dr Cláudia Lobato da Silva/ André Branco
Spain	Cell Reprogramming and Manufacturing Unit (UPRC), Andalusian Network for Design and Translation of Advanced Therapies (AND&TAT) Rafael Campos-Cuerva (Campos-Cuerva, R) / Blanca Arribas-Arribas (Arribas-Arribas, B)
Sweden	Next Cell Pharma AB Leo Groenewegen/ Johanna Dahllund/ Bahareh Khalaj
UK	The Francis Crick Institute (Bonnet Lab) Dr Sara Ali/ Dr Roosa Harmo
UK	Spinal Studies & Cartilage Research Group Professor Sally Roberts/ Dr Claire Meehan /John Garcia
UK	Mesenchymal Stem Cells group, University of Leeds

	Prof. Elena Jones/ Dr. Jehan El-Jawhari
UK	NHBST Oxford Stem Cells and Immunotherapies Lab Professor John Girdlestone
UK	University of York Department of Biology Professor Paul Genever / Dr Alasdair Gawain Kay
UK	National Institute of Biological Standards and Controls. Dr. Sandrine Vessillier/ Dr. Deepa Rajagopal
USA	CBER Office of Tissues and Advanced Therapies U.S. Food and Drug Administration Dr. Steve Bauer/ Dr. Heba Degheidy

Appendix 2: Antibodies used by participants. Antibodies marked with * are the statutory markers given by the ISCT for MSC identity.

Table 2:

Antibody	**Supplier**	**Lab codes**
CD90*	BD Biosciences	1, 6, 7, 11 and 15
	Beckman Coulter	2 and 10
	Miltenyi Biotec	8 and 13
	Ebioscience	3
	BioLegend	4
	BD Stemflow	9
	BD Pharmingen	12
CD105*	BioLegend	1, 3, 4, 12
	Beckman Coulter	2 and 10
	BD Bioscience	6, 7, 11 and 15
	Miltenyi Biotec	8 and 13
	BD Stemflow	9
CD73*	BD Biosciences	1, 3, 6, 7, 8, 11 and 15
	BD Pharmigen	2
	BioLegend	4 and 12
	BD Stemflow	9
	Beckman Coulter	10
	Miltenyi Biotec	13
CD44	BioLegend	1, 4, and 6

	BD Stemflow	9
CD29	BD Biosciences	3
CD142	Miltenyi Biotec	10
CD146	*Miltenyi Biotec*	8, and 10
CD271	Biolegend	3
CD45*	BioLegend Invitrogen BD Biosciences *Miltenyi Biotec* Beckman Coulter BD Pharminogen	1, 2, 4, and 12 3 7 8 10 11
CD34*	BD Biosciences Biolegend Beckman Coulter BD Pharminogen	1, 3, and 7 2, 4, and 12 10 11
CD14*	BioLegend BD Biosciences *Miltenyi Biotec* Beckman Coulter	1 7 8 10
CD19*	BD Biosciences Biolegend Beckman Coulter	1 2 10
HLA-DR*	*BioLegend* *BD Biosciences*	1, 2, 4, and 12 3, 7, 8

	Miltenyi Biotec	13
Negative Cocktail	BD Bioscience BD Stemflow *Miltenyi Biotec*	6, 11 and 15 9 13
CD11b	BD Biosciences Biolegend BD Pharminogen	1, and 3 2, and 4 11
CD31	Biolegend Beckman Coulter	2 10
CD80	BioLegend	1
CD20	*Miltenyi Biotec*	13
MHC Class I	Biolegend	2

Table 3: Flow Cytometer systems used by participants.

Flow Cytometer	**Lab Codes**
BD FACSCalibur	1
MACS Quant Analyzer	2 and 13
Attune Acoustic Focusing Cytometer	8
Cytoflex	10
Accuri C6 Plus	9
Beckman Coulter, FC500 Beckman Coulter Navios	4 12
BD Canto II	6, 7, 11 and 15
LSRIIa	3

Table 4 : Flow cytometers used by participants

Analysis Software	**Lab Codes**
FlowJo	1, 2, 3, 4, 6,10, 11 and 15
Attune Cytometric Software	8
BD CSampler Analysis Software	9
FACSDIVA	7
Beckman Coulter Navios	12
MACSQuantify Software	13

Appendix 3. Comments on the report from participants

All 15 participants were sent a draft report and asked to comment on the content and conclusions, and to confirm that their results had been reported correctly. Eight participants responded (55%) and all agreed with the findings of the report and the following specific comments were received:

Lab 1"Thank you for sending us the draft report. We have included a comment concerning CD80 expression and just corrected one typo. Thank you for the collaboration. We noticed that data from CD80 is not present as part of the negative markers".

Response: CD80 data added to report.

Lab 2

"Thank you for the summary report. Our affiliation has changed recently, and my position is not Professor. Affiliation and staff members are: XX. I hope results provided have been useful and do not hesitate to count on us for future studies".
Response: Correction made in report.

Lab 4

"Could you add 'X' to my affiliation?"

Response: Changed in the report

Lab 6

"Thanks for sending this and coordinating the effort. I have included a version with track changes throughout. I think it important to clarify that the MSCs were not tested to all the ISCT criteria since they are intended as a flow cytometry reference material. I also think it

important to discuss the ISCT criteria a bit more and to compare the outcome of this study to those criteria".

Response: Comments added to report

"I used Dr. X's version to add my comments/edits. In addition, to Dr. X's recommendations in the email below, it would be useful to include more details/ explanations in the discussion section for the reported CD90, CD105, and CD73 values that were below the ISCT recommendations >95%. Specifically, for CD90 values that were around 50%".

Response: Comments added to report

"Content/comments added to report: Two additional markers, CD45 and CD29 were included because they are often characterized on MSC preparations (reference). Since this material is intended for use in flow cytometry studies, no additional characterization of this MSC line is reported. (With regards to figure 1) It would useful and informative to show the results from the control MSC and NSC lines as well. I agree the comparability to the BM and cord blood derived MSC lines and the NSC would be useful information to show".

Response: Comments added to report and data for control MSC and NSC shown in figure 1.

Lab 7

(Additional name to lab) - I helped with the analysis and I am in the spinal studies group UK.

Response: Changed in the report.

"Consider stromal instead of stem. If you do not replace 'stem' with 'stromal' throughout, I think it should definitely be described as that as an alternative here at the beginning".

Response: Comment was added to say that they can also be referred to as stromal cells.

"I think they stipulate levels (ie >95percent for the +ve markers and <2percent for the negative ones)".

Response: Added in report

"There are options for the negative markers, so this probably needs correcting. CD11b or CD14".

Response: this was not changed as although you can choose which markers to test, ultimately MSCs should be negative for both/all markers.

“With regards to materials and methods: Do you not need to add suppliers? Most journals request this so should this not follow that convention too? And the same for all reagents?”

Response: not relevant for this report.

“With regards to differentiation protocol used: How were these shown to be differentiated? (or can quote a paper where you have described this).”

Response: Reference added.

With regards to human BM and Umbilical MSCs used: I think these need much more detail on them.

Response: no more detail to add, they are human MSC lines used as controls.

“As you’ve used 11b it does need to be clearer in the introduction. Did you test CD14? Shouldn’t you also include details of your set up (software, labels etc) as are reported for the other 15 sites?”

Response: Info is in the report

“Presumably this ‘average’ is a mean if you’re quoting SDs?”

Response: Changed in the report.

“With regards to vials: Would it be worth saying how they were reconstituted here?”

Response: Added in the report.

“It would be good to mention that the three vials were run as separate samples by all research groups. It would also be interesting to see the variation in results for those repeats and CVs. Can these results be included?”

Response: Info is in the report

“With regards to CD271 as a marker: This marker is lost in culture on bone marrow derived MSCs. I can find a reference for this if needed”.

Response: Added in the report.

“With regards to CD45 result that was excluded: Why was it excluded?”

Response: Info is in the report

“What were the stability studies and how were they assessed?”

Response: Added in the report.

“With regards to problems with low cell numbers: Did you not say that this problem might have been something to do with the aliquoting your end?”

Response: We checked cell numbers based on our flow data and deemed there to be sufficient numbers in all the vials we tested on site.

Lab 9

“Attached with only one change from our side (added X to our list)”

Response: Correction made on report

Lab 11

“One small change in my name”.

Response: Changed in the report.

Lab 12

“I'm only a Dr, not a professor. It looks as if we were lab 12 and excluded. It would be useful to know why our vials did not reconstitute as expected”.

Response: Changed in the report. Participant advised low numbers were due to protocol used to reconstitute.

Appendix 4: Safety testing of starting material

Biological Safety					
Test		Assay	Acceptance Criteria	Result	
EP Sterility		Bacteria/fungi	Not detected	☒ Pass	☐ Not Tested
Mycoplasma		Culture	Not detected	☒ Pass	☐ Not Tested
Mycoplasma		PCR	Not detected	☒ Pass	☐ Not Tested
Virus Panel	Hepatitis B	PCR	Not Detected	☒ Pass	☐ Not Tested
	Hepatitis C	PCR	Not Detected	☒ Pass	☐ Not Tested
	HIV-1	PCR	Not Detected	☒ Pass	☐ Not Tested
	HIV-2	PCR	Not Detected	☐ Pass	☒ Not Tested
	HTLV-1	PCR	Not Detected	☒ Pass	☐ Not Tested
	Epstein Barr virus (EBV)	PCR	Not Detected	☒ Pass	☐ Not Tested
	Human Cytomegalovirus CMV)	PCR	Not Detected	☒ Pass	☐ Not Tested